JC11 Rec'd PCT/PTO 09 MAR 2001

FORM PTO-1390 U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/ES/US) CONCERNING A FILING UNDER 35 U.S.C 371	1588.GLE.PT
	U S APPLICATION NO (If known, see 37 CFR 1 5) 09/786835

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/06692	11 September 1999	10 September 1998

TITLE OF INVENTION

REGULATORY SEQUENCES FOR ROOT SPECIFIC OR ROOT ABUNDANT GENE EXPRESSION IN PLANTS

APPLICANT(S) FOR DO/EO/US

Günter Feix, Doerte Wulff

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☒ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 10 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    - Certificate of Mailing
    - Postcard

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U.S. APPLICATION NO (if known, see 37 CFR 1.5) 09/786835	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER

				CALCULATIONS	PTO USE ONLY
17. ☐ The following fees are submitted: **BASIC NATIONAL FEE (37 CFR 1.492 (a)(1) - (5)):** Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO . . . .**$970.00** International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO. . . . . .**$840.00** International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO . . . . **$690.00** International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4). . . . . **$670.00** International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4). **$96.00**					
ENTER APPROPRIATE BASIC FEE AMOUNT =				840.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).					
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	**25-20 =**	**5**	**X $18.00**	$ 90.00	
Independent claims	**-3 =**		**X $78.00**	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $260.00	$ 260 00	
TOTAL OF ABOVE CALCULATIONS =				$1190.00	
Reduction of ½ for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$1190.00	
				Amount to be refunded:	$
				charged:	$

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a. ☒ A credit card authorization in the amount of $1190.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 50-0881 in the amount of $__________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge additional fees which may be required, or credit any overpayment to Deposit Account No. 50-0881. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

Randall B. Bateman
MORRISS, BATEMAN, O'BRYANT & COMPAGNI
5882 South 900 East, Suite 300
Salt Lake City, Utah 84121
(801) 685-2302 phone
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Randall Bateman

SIGNATURE

Randall B. Bateman

NAME

37,774

REGISTRATION NUMBER

CERTIFICATE OF DEPOSIT UNDER 37 C.F.R. § 1.10

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail, postage prepaid, under 37 C.F.R. § 1.10 on the date indicated below and is addressed to Asst. Commissioner for Patents & Trademarks, Washington, D.C. 20231.

Randall Bateman
Randall B. Bateman

March 9, 2001
Date

EXPRESS MAIL NO. EL 593621462 US

13 Rec'd PCT/PTO 11 APR 2001
09/786835

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Günter Feix et al.

Serial Number: ______________(National Stage of PCT/EP99/06692

Filed: 10 September 1999

Group: ______________

Examiner: ______________

For: REGULATORY SEQUENCES FOR ROOT SPECIFIC OR REOOT ABUNDANT GENE EXPRESSION IN PLANTS

Attorney Docket: 1588.GLE.PT

Commissioner of Patents
and Trademarks
Washington, D. C. 20231

I hereby certify that this correspondence is being deposited with the United States Postal Service via First Class Mail addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on April 3, 2001
(Date of Deposit)

Randall Baler

PRELIMINARY AMENDMENT

Dear Sir:

Prior to examination of the above-referenced application, please enter the following amendment.

In The CLAIMS.

Please cancel claim 1 through 29 and replace therefor, new claims 30 through 68 as set forth in the replacement sheets attached hereto.

A credit card authorization to cover the additional claim fees is also included.

Applicant submits that the application is believed to be in condition for allowance. Should the Examiner believe that any adverse action is necessary, it is requested that he contact Randall B. Bateman at (801) 6685-2302 so that such matters may be resolved as expeditiously as possible.

Respectfully Submitted,

MORRISS, BATEMAN, O'BRYANT & COMPAGNI, PC

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CLAIMS

What is claimed is:

30. (New) A nucleic acid sequence for use in cloning and expressing a root specific nucleic acid sequence in a plant, selected from the group consisting of: a nucleic acid sequence of SEQ ID No. 1; a nucleic acid sequence complementary to SEQ ID No. 1; a nucleic acid sequence having a degree of identity of more than 70% to SEQ ID No. 1 or to the sequence complementary to SEQ ID No. 1; alleles of the nucleic acid sequence of SEQ ID No. 1; alleles of the complementary sequence; alleles of a nucleic acid sequence having a degree of identity of more than 70% to SEQ ID No. 1; and alleles of a nucleic acid sequence having a degree of identity of more than 70% to a sequence complementary to SEQ ID No. 1.

31.(New) A nucleic acid sequence for use in cloning and expressing a root specific nucleic acid sequence in a plant, selected from the group consisting of: a nucleic acid sequence of SEQ ID No. 2; a nucleic acid sequence complementary to SEQ ID No. 2; a nucleic acid sequence having a degree of identity of more than 70% to SEQ ID No. 2 or to the sequence complementary to SEQ ID No. 2; alleles of the nucleic acid sequence of SEQ ID No. 2; alleles of the complementary sequence; alleles of a nucleic acid sequence having a degree of identity of more than 70% to SEQ ID No. 2; and alleles of a nucleic acid sequence having a degree of identity of more than 70% to a sequence complementary to SEQ ID No. 2.

32. (New) A nucleic acid sequence for use in cloning and expressing a root specific nucleic acid sequence in a plant, selected from the group consisting of: SEQ ID Nos. 3-11; a nucleic acid sequence complementary to any of SEQ ID Nos. 3-11; a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 3-11; a nucleic acid sequence having a degree of identity of more than 70% to a sequence complementary to any of SEQ ID Nos. 3-11; alleles of the nucleic acid sequence of any of SEQ ID Nos. 3-11; alleles of a sequence complementary to any of SEQ ID Nos. 3-11; alleles of a nucleic acid sequence having a degree of

identity of more than 70% to any of SEQ ID Nos. 3-11; and alleles of a nucleic acid sequence having a degree of identity of more than 70% to a sequence complementary to any of SEQ ID Nos. 3-11.

33. (New) A nucleic acid sequence for use in cloning and expressing a root specific nucleic acid sequence in a plant, selected from the group consisting of: SEQ ID Nos. 12-15; a nucleic acid sequence complementary to any of SEQ ID Nos. 12-15; a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 12-15; a nucleic acid sequence having a degree of identity of more than 70% to a sequence complementary to any of SEQ ID Nos. 12-15; alleles of the nucleic acid sequence of any of SEQ ID Nos. 12-15; alleles of a sequence complementary to any of SEQ ID Nos. 12-15; alleles of a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 12-15; and alleles of a nucleic acid sequence having a degree of identity of more than 70% to a sequence complementary to any of SEQ ID Nos. 12-15.

34. (New) A nucleic acid sequence derived from maize for use in cloning and expressing a root specific nucleic acid sequence in a plant, the sequence being selected from the group consisting of: a nucleic acid sequence of any of SEQ ID Nos. 1-15; a nucleic acid sequence complementary to any of SEQ ID Nos. 1-15; a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15 or to a sequence complementary to any of SEQ ID Nos. 1-15; alleles of the nucleic acid sequence of any of SEQ ID Nos. 1-15; alleles of a sequence complementary to any of SEQ ID Nos. 1-15; alleles of a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15; and alleles of a nucleic acid sequence having a degree of identity of more than 70% to a sequence complementary to any of SEQ ID Nos. 1-15.

35.(New) A vector comprising a nucleic acid sequence selected from the group consisting of:

a nucleic acid sequence of any of SEQ ID Nos. 1-15; a nucleic acid sequence complementary to any of SEQ ID Nos. 1-15; a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15 or to a sequence complementary to any of SEQ ID Nos. 1-15; alleles of the nucleic acid sequence of any of SEQ ID Nos. 1-15; alleles of a sequence complementary to any of SEQ ID Nos. 1-15; alleles of a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15; and alleles of a nucleic acid sequence having a degree of identity of more than 70% to a sequence complementary to any of SEQ ID Nos. 1-15.

36.(New) The vector of Claim 35, which is a bacterial or viral vector.

37.(New) The vector of Claim 35, wherein the nucleic acid sequence is operably linked to a gene of interest.

38. (New) The vector of Claim 37, wherein the gene of interest is a gene which confers a character of agronomic or industrial benefit.

39. (New) The vector of Claim 38, wherein the gene of interest is a gene for resistance to infection by a virus, a gene conferring resistance to an herbicide or insecticide, or a gene whose expression confers male sterility.

40.(New) The vector of Claim 37, which further includes regulatory elements directing or enhancing the expression of the gene of interest.

41.(New) The vector of Claim 40, wherein the regulatory elements are 5', 3', or 5' and 3' elements.

42. (New) The vector of Claim 41, wherein the 3' regulatory element is a poly A addition sequence of an NOS gene of Agrobacterium tumefaciens.

43.(New) The vector of Claim 40, which further includes T-DNA.

44.(New) The vector of Claim 43, which includes the left border of T-DNA, the right border of T-DNA, or both the left and right borders of T-DNA.

45. (New) The vector of Claim 43, wherein the nucleic acid sequence is located within the T-DNA or adjacent to the T-DNA.

46.(New) The vector of Claim 45, wherein the nucleic acid sequence is in conjunction with the gene of interest and is located within the T-DNA or adjacent to it.

47.(New) A host cell containing a vector comprising a nucleic acid sequence selected from the group consisting of:
a nucleic acid sequence of any of SEQ ID Nos. 1-15; a nucleic acid sequence complementary to any of SEQ ID Nos. 1-15; a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15 or to a sequence complementary to any of SEQ ID Nos. 1-15; alleles of the nucleic acid sequence of any of SEQ ID Nos. 1-15; alleles of a sequence complementary to any of SEQ ID Nos. 1-15; alleles of a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15; and alleles of a nucleic acid sequence having a degree of identity of more than 70% to a sequence complementary to any of SEQ ID Nos. 1-15.

48. (New) The host cell of Claim47, wherein the nucleic acid sequence is operably linked to a gene of interest, and the gene of interest is a gene which confers a character of agronomic or industrial benefit.

49.(New) The host cell of Claim 48, wherein the gene of interest is a gene for resistance to infection by a virus, a gene conferring resistance to an herbicide or insecticide, or a gene whose expression confers male sterility.

50.(New) The host cell of Claim 48, which further includes regulatory elements directing or enhancing the expression of the gene of interest.

51.(New) The host cell of Claim 50, wherein the regulatory elements are 5', 3', or 5' and 3' elements.

52.(New) The host cell of Claim 51, wherein the 3' regulatory element is a poly A addition sequence of an NOS gene of Agrobacterium tumefaciens.

53.(New) The host cell of Claim 50, which further includes T-DNA.

54.(New) The host cell of Claim 53, which includes the left border of T-DNA, the right border of T-DNA, or both the left and right borders of T-DNA.

55. (New) The host cell of Claim 54, wherein the nucleic acid sequence is located within the T-DNA or adjacent to the T-DNA.

56. (New) The host cell of Claim 53, wherein the nucleic acid sequence is in conjunction with the gene of interest and is located within the T-DNA or adjacent to it.

57.(New) The host cell of Claim 47, which is a plant, yeast, or bacterial cell.

58.(New) The host cell of Claim 57, which is a cell from a monocotyledonous or dicotyledonous plant.

59.(New) A cell culture including a host cell selected from the group consisting of plant, yeast and bacterial cells; said host cell containing a vector comprising a nucleic acid sequence selected from the group consisting of:
a nucleic acid sequence of any of SEQ ID Nos. 1-15; a nucleic acid sequence complementary to any of SEQ ID Nos. 1-15; a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15 or to a sequence

complementary to any of SEQ ID Nos. 1-15; alleles of the nucleic acid sequence of any of SEQ ID Nos. 1-15; alleles of a sequence complementary to any of SEQ ID Nos. 1-15; alleles of a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15; and alleles of a nucleic acid sequence having a degree of identity of more than 70% to a sequence complementary to any of SEQ ID Nos. 1-15.

60.(New) A plant including a host cell containing a vector comprising a nucleic acid sequence selected from the group consisting of:
a nucleic acid sequence of any of SEQ ID Nos. 1-15; a nucleic acid sequence complementary to any of SEQ ID Nos. 1-15; a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15 or to a sequence complementary to any of SEQ ID Nos. 1-15; alleles of the nucleic acid sequence of any of SEQ ID Nos. 1-15; alleles of a sequence complementary to any of SEQ ID Nos. 1-15; alleles of a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15; and alleles of a nucleic acid sequence having a degree of identity of more than 70% to a sequence complementary to any of SEQ ID Nos. 1-15.

61.(New) A composition of matter comprising seeds, propagation material, harvest material, or plant tissue, wherein the composition includes a host cell containing a vector comprising a nucleic acid sequence selected from the group consisting of:
a nucleic acid sequence of any of SEQ ID Nos. 1-15; a nucleic acid sequence complementary to any of SEQ ID Nos. 1-15; a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15 or to a sequence complementary to any of SEQ ID Nos. 1-15; alleles of the nucleic acid sequence of any of SEQ ID Nos. 1-15; alleles of a sequence complementary to any of SEQ ID Nos. 1-15; alleles of a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15; and alleles of a nucleic acid sequence having a degree of identity of more than 70% to a sequence complementary to any of SEQ ID Nos. 1-15.

degree of identity of more than 70% to a sequence complementary to any of SEQ ID Nos. 1-15.

62.(New) A composition of matter comprising seeds, propagation material, harvest material, or plant tissue derived from a plant having a host cell containing a vector comprising a nucleic acid sequence selected from the group consisting of:
a nucleic acid sequence of any of SEQ ID Nos. 1-15; a nucleic acid sequence complementary to any of SEQ ID Nos. 1-15; a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15 or to a sequence complementary to any of SEQ ID Nos. 1-15; alleles of the nucleic acid sequence of any of SEQ ID Nos. 1-15; alleles of a sequence complementary to any of SEQ ID Nos. 1-15; alleles of a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15; and alleles of a nucleic acid sequence having a degree of identity of more than 70% to a sequence complementary to any of SEQ ID Nos. 1-15.

63.(New) A method of genetically modifying a cell, comprising a step of transforming the cell with a nucleic acid sequence selected from the group consisting of:
a nucleic acid sequence of any of SEQ ID Nos. 1-15; a nucleic acid sequence complementary to any of SEQ ID Nos. 1-15; a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15 or to a sequence complementary to any of SEQ ID Nos. 1-15; alleles of the nucleic acid sequence of any of SEQ ID Nos. 1-15; alleles of a sequence complementary to any of SEQ ID Nos. 1-15; alleles of a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15; and alleles of a nucleic acid sequence having a degree of identity of more than 70% to a sequence complementary to any of SEQ ID Nos. 1-15;
wherein a gene of interest contained in the vector is expressible in the cell.

64.(New) The method of Claim 63, wherein the cell is a plant, bacterial, or yeast cell.

65. (New) The method of Claim 63, containing a further step of regenerating the transformed cell to produce a differentiated plant.

66.(New) The method of Claim 63, wherein the step of transforming is accomplished by transfer of a vector or of nucleic acid sequences from a bacterium to the cell.

67. (New) The method of Claim 63, wherein the step of transforming of the cell further includes either a step of microinjection with a vector or nucleic acid sequence or a step of particle bombardment with a vector or nucleic acid sequence, such that the step of transforming is accomplished by direct intake of a vector or nucleic acid sequence.

68. (New) A method for isolating a root specific gene and/or a root specific regulatory element from a plant, in which nucleic acid sequences derived from the plant are screened using a nucleic acid sequence selected from the group consisting of : a nucleic acid sequence of any of SEQ ID Nos. 1-15; a nucleic acid sequence complementary to any of SEQ ID Nos. 1-15; a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15 or to a sequence complementary to any of SEQ ID Nos. 1-15; alleles of the nucleic acid sequence of any of SEQ ID Nos. 1-15; alleles of a sequence complementary to any of SEQ ID Nos. 1-15; alleles of a nucleic acid sequence having a degree of identity of more than 70% to any of SEQ ID Nos. 1-15; alleles of a nucleic acid sequence having a degree of identity of more than 70% to a sequence complementary to any of SEQ ID Nos. 1-15; and a vector comprising any of the preceding nucleic acid sequences and alleles.

06-19-01

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09/786835

SEQ

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Günter Feix et al.

Serial Number: 09/786,835 (National Stage of PCT/EP99/06692)

Filed: 10 September 1999

Group: ______________

Examiner: ______________

For: REGULATORY SEQUENCES FOR ROOT SPECIFIC OR REOOT ABUNDANT GENE EXPRESSION IN PLANTS

Attorney Docket: 1588.GLE.PT

Assistant Commissioner for Patents
Washington, D. C. 20231

I hereby certify that this correspondence is being deposited with the United States Postal Service via Express Mail addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on June 19, 2001
(Date of Deposit)

Sequence Listing under 37 CFR 1.82(g)

Statement under 37 CFR 1.821(f)

A computer-readable copy of the Sequence Listing for the above-referenced case is hereby submitted under the provisions of 37 CFR 1.821-1.825.

Applicant hereby states that the information recorded in computer-readable form is identical to that of the paper copy filed with the application.

Applicant further states that the Sequence Listing contains no new subject matter.

Respectfully submitted this 19th day of June, 2001.

Morriss, Bateman, O'Bryant & Compagni

Randall B. Bateman
Reg. No. 37,774

09/786835
JC02 Rec'd PCT/PTO 0 9 MAR 2001

5|PRTS

Description

The present invention relates to isolated regulatory elements, in particular promoters and 3' regulatory elements, that drive root specific or root abundant expression in plants, in particular in monocotyledonous plants, to vectors containing the regulatory elements, to host cells containing the vectors, to plants containing the host cells and to methods for isolating regulatory elements that contribute to tissue-preferred gene expression in plants.

Gene expression is considered to comprise a number of steps from the DNA to the final protein product. Initiation of transcription of a gene is generally believed to be the predominant controlling factor in determining expression of a gene. The transcriptional controls are generally located in relatively short sequence elements embedded in the 5'-flanking and/or 3'-flanking region of the transcribed gene with which DNA-binding proteins may interact. These DNA sequence elements serve to promote the formation of transcriptional complexes and eventually initiate gene expression processes. It is furthermore known that the regulation of gene expression often depends upon the development stage and the tissue specificity of the cell concerned. Thus, certain tissues of organisms such as plants may exhibit a metabolism and a protein composition different from other tissues of the plant or different from the same tissue in a different developmental stage.

Controlling the expression of genes in transgenic plants is considered to have high commercial value. The ability to control gene expression is useful for conferring resistance and immunity to certain diseases or to modify the metabolism of the tissue. The transfer of heterologous genes or genes of interest into a plant under control of tissue-specific genes provides a powerful means of conferring selective advantages to plants and to increase their commercial value.

It is also considered particularly important to develop transcriptional regulatory units that direct gene expression selectively to root tissue. Root preferred gene expression will provide several advantages to a plant, such as alteration of the function of the root tissue; modification of the growth rate; resistance to root preferred pathogens, pests, herbicides or adverse weather conditions; as well as broadening the range of soils or environments in which said plant may grow. Root abundant or root specific gene expression would provide a mechanism according to which morphology and metabolism may be altered to improve the yield and to produce useful proteins in greater amounts.

Thus, there is a need to provide regulatory elements capable of directing transcription specifically in root tissue. WO97/44448 describes promoter elements conferring root preferred gene expression. The disclosed promoter elements were obtained from maize seedlings. However, the isolated promoter elements may not be suitable for any desired transformation problem.

Furthermore, WO94/02619 discloses regulatory sequences for root specific or root abundant gene expression derived from Brassica sp. However, this promoter was not shown to work in monocotyle-

donous plants with an efficiency great enough to provide means for commercial use.

Thus, the technical problem underlying the present invention is to provide novel regulatory elements for use in cloning and expressing root specific or root abundant genes, in particular for use in monocotyledonous plants which provide a high expression efficiency together with a high tissue specificity.

The present invention solves the technical problem underlying the present invention by providing purified and isolated new nucleic acid sequences for use in cloning and expressing a root specific or root abundant gene in a plant which are selected from the group consisting of

(a) the nucleic acid sequence set out in SEQ ID No. 1 or a part or complementary strand thereof,

(b) a nucleic acid sequence which hybridizes to the nucleic acid sequence defined in SEQ ID No. 1 or a complementary strand thereof and

(c) alleles or derivatives of the nucleic acid sequence defined in (a) or (b) or a complementary strand thereof.

In particular, the problem is solved by providing a purified nucleic acid sequence according to the above wherein the sequence is selected from the group consisting of (a1) the nucleic acid sequence set out in SEQ ID No. 2 or a part or complementary strand thereof, (b1) a nucleic acid sequence which hybridizes to the nucleic acid sequence defined in SEQ ID No. 2 and (c1) alleles or derivatives of the nucleic acid sequence defined in (a1) or (b1).

In a further particularly preferred embodiment, the problem is solved by providing a purified nucleic acid sequence according to the above which is selected from the group consisting of (a2) the nucleic acid sequence set out in any one of SEQ ID Nos. 3 to 11, (b2) a nucleic acid sequence which hybridizes to any one of the nucleic acid sequences defined in any one of SEQ ID Nos. 3 to 11 and alleles or derivatives of the nucleic acid sequence defined in (a2) or (b2).

The invention also relates to purified nucleic acid sequences for use in cloning and expressing a root specific or root abundant gene in a plant that are selected from the group consisting of (a3) the nucleic acid sequence set out in any one of SEQ ID No. 12, 13, 14 or 15 or a part or complementary strand thereof, (b3) a nucleic acid sequence hybridizing to any one of the sequences defined in (a3) and (c3) alleles or derivatives of the nucleic acid sequences defined in (a3) or (b3).

The sequences set out in SEQ ID No. 1 to 11 comprise isolated 5' regulatory elements and are considered to be promoters or parts thereof, i.e. promoter elements. These sequences are capable of modulating, initiating and/or contributing to the transcription of nucleic acid sequences operably linked to them. In a preferred embodiment of the present invention these sequences may additionally contain at their 3' terminus the nucleotide sequence from position 1761 to 1780 as depicted in SEQ ID No. 14, which is CCTGGACTCG CTCACTGGCA.

The sequences set out in SEQ ID No. 12, 13 and 15 comprise isolated 3' regulatory elements and are considered to be 3' transcription regulatory elements, or parts thereof, for instance polyadenylation signals. The sequences of SEQ ID Nos. 12 and 15

linked in 5' to 3' direction to each other represent the complete 3' regulatory element of the present invention.

These sequences are capable of modulating, in particular contributing to, or terminating the transcription of nucleic acid sequences operably linked to them.

The sequence set out in SEQ ID No. 14 is a genomic clone comprising all of the above sequences except the 3' regulatory element of the SEQ ID No. 15.

The present invention also relates to nucleic acid sequences which hybridize, in particular under stringent conditions, to the sequences set out in any one of SEQ ID No. 1 to 15. In particular, these sequences have a degree of identity of 70% to the sequence of SEQ ID Nos. 1 to 15.

In the context of the present invention, nucleic acid sequences which hybridise to any one of the specifically disclosed sequences of SEQ. Id. Nos. 1 to 15 are sequences which have a degree of 60% to 70% sequence identity to the specifically disclosed sequence on nucleotide level. In an even more preferred embodiment of the present invention, sequences which are encompassed by the present invention are sequences which have a degree of identity of more than 70% or 80% and even more preferred more than 90%, 95% or 99% to the specifically disclosed sequences on nucleotide level.

The present invention also relates to nucleic acid sequences which encode proteins wherein the amino acid sequence of the proteins have a degree of identity of 66% to 90% on amino acid level, most preferably a degree of identity of more than 90%, 95% or 99% identity on amino acid level.

Thus, the present invention relates to nucleic acid sequences, in particular DNA sequences which hybridise under the following conditions to the sequences specifically disclosed:

Hybridisation buffer: 1 M NaCl; 1% SDS; 10% dextran sulphate; 100 μg/ml ssDNA
Hybridisation temperature: 65° C
First wash: 2 x SSC; 0.5% SDS at room temperature
Second wash: 0.2 x SSC; 0.5% SDS at 65°C.

More preferably, the hybridisation conditions are chosen as identified above, except that a hybridisation temperature and second wash temperature of 68° C, and even more preferred, a hybridisation temperature and second wash temperature of 70° C is applied.

Thus, the present invention also comprises functionally equivalent nucleic acid sequences to the sequences of any one of SEQ ID No. 1 to 15, in particular sequences which have at least homology to the sequence of SEQ ID No. 1 to 15. The invention also relates to alleles and derivatives of the sequences mentioned above which are defined as sequences being essentially similar to the above sequences but comprising, for instance, nucleotide exchanges, substitutions (also by unusual nucleotides), rearrangements, mutations, deletions, insertions, additions or nucleotide modifications and are functionally equivalent to the sequences set out in SEQ ID No. 1 to 15.

The nucleic acid sequences of the present invention are, in a preferred embodiment, derived from maize (Zea mays), most preferably from maize roots.

The nucleic acid sequences of the present invention are useful for cloning tissue specific, in particular root specific nucleic acid sequences, in particular regulatory elements and/or genes, in plants, in particular in monocotyledonous plants. Thus, the present invention provides the means for isolation of transcription regulatory elements that direct or contribute to tissue-preferred gene expression in plants, in particular in monocotyledonous plants, such as maize. The present invention also provides the means of isolating tissue specifically expressed genes, in particular root specifically expressed genes and their transcripts.

The nucleic acid sequences of the present invention are also useful for expressing genes of interest in plants, in particular in the roots of plants and especially in the roots of monocotyledonous plants such as maize or of dicotyledonous plants such as sugar beets (Beta vulgaris). Thus, the present invention provides the means to direct the expression of a gene of interest in a tissue-specific or tissue-abundant manner in roots, for instance in roots of seedlings. Accordingly, the proteins encoded by the gene of interest can be accumulated in roots. For instance, the promoter of the present invention is particularly useful in driving the transcription of heterologous structural genes that confer disease immunity or resistance to disease-susceptible roots.

In the context of the present invention, a number of terms shall be utilized as follows.

In the context of the present invention the term "disease" encompasses any adverse condition caused to a plant by a virus or

an organism other than itself, such as fungi, bacteria and insects.

The term "promoter" refers to a sequence of DNA, usually upstream (5') to the coding sequence of a structural gene, which controls the expression of the coding region by providing the recognition for RNA polymerase and/or other factors required for transcription to start at the correct site. Promoter sequences are necessary, but not always sufficient to drive the expression of the gene. A "promoter element" constitutes a fraction of the DNA sequence of the promoter.

A "3' regulatory element (or "3' end") refers to that portion of a gene comprising a DNA segment, excluding the 5' sequence which drives the initiation of transcription and the structural portion of the gene, that determine the correct termination site and contains a polyadenylation signal and any other regulatory signals capable of effecting messenger RNA (mRNA) processing or gene expression. The polyadenylation signal is usually characterized by effecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. Polyadenylation signals are commonly recognised by the presence of homology to the canonical form 5'-AATAA-3', although variations are not uncommon.

"Nucleic acid" refers to a large molecule which can be single or double stranded, composed of monomers (nucleotides) containing a sugar, phosphate and either a purine or pyrimidine. The nucleic acid may be cDNA, genomic DNA, or RNA, for instance mRNA.

The term "nucleic acid sequence" refers to a natural or synthetic polymer of DNA or RNA which may be single or double stranded, alternatively containing synthetic, non-natural or al-

tered nucleotide bases capable of incorporation into DNA or RNA polymers.

The term "gene" refers to a DNA sequence that codes for a specific protein and regulatory elements controlling the expression of this DNA sequence.
The term "regulatory element" refers to a sequence located upstream (5'), within and/or downstream (3') to a coding sequence whose transcription and expression is controlled by the regulatory element, potentially in conjunction with the protein biosynthetic apparatus of the cell. "Regulation" or "regulate" refer to the modulation of the gene expression induced by DNA sequence elements located primarily, but not exclusively upstream from (5') the transcription start of the gene of interest. Regulation may result in an all or none response to a stimulation, or it may result in variations in the level of gene expression.

The term "coding sequence" refers to that portion of a gene encoding a protein, polypeptide, or a portion thereof, and excluding the regulatory sequences which drive the initiation or termination of transcription. The coding sequence or the regulatory element may be one normally found in the cell, in which case it is called "autologous", or it may be one not normally found in a cellular location, in which case it is termed a "heterologous gene" or "heterologous nucleic acid sequence". A heterologous gene may also be composed of autologous elements arranged in an order and/or orientation not normally found in the cell in which it is transferred. A heterologous gene may be derived in whole or in part from any source known to the art, including a bacterial or viral genome or episome, eukaryotic nuclear or plasmid DNA, cDNA or chemically synthesized DNA. The structural gene may constitute an uninterrupted coding region or

it may include one or more introns bounded by appropriate splice junctions. The structural gene may be a composite of segments derived from different sources, naturally occurring or synthetic.

The term "vector" refers to a recombinant DNA construct which may be a plasmid, virus, or autonomously replicating sequence, phage or nucleotide sequence, linear or circular, of a single or double stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell, in particular a plant cell.

As used herein, "plant" refers to photosynthetic organisms, such as whole plants including algae, mosses, ferns and plant-derived tissues. "Plant derived tissues" refers to differentiated and undifferentiated tissues of a plant, including roots, shoots, shoot meristems, coleoptilar nodes, tassels, leaves, cotyledondous petals, pollen, ovules, tubers, seeds, kernels and various forms of cells in culture such as intact cells, protoplasts, embryos and callus tissue. Within the context of the present invention, the term "root" includes various groups of primary, lateral seminal or crown roots, and root tips. Plant-derived tissues may be in planta, or in organ, tissue or cell culture. A "monocotyledonous plant" refers to a plant whose seeds have only one cotyledon, or organ of the embryo that stores and absorbs food. A "dicotyledonous plant" refers to a plant whose seeds have two cotyledons.

As used herein, "transformation" refers to the process by which cells, tissues or plants acquire properties encoded on a nucleic

acid molecule that has been transferred to the cell, tissue or plant.

"Transformation" and "transferring" refers to methods to transfer DNA into cells including, but not limited to, particle bombardment, microinjection, permeabilizing the cell membrane with various physical (e.g., electroporation) or chemical (e.g., polyethylene glycol, PEG) treatments.

The term "host cell" refers to a cell which has been genetically modified by transfer of a heterologous or autologous nucleic acid sequence or its descendants still containing this sequence. These cells are also termed "transgenic cells". In the case of an autologous nucleic acid sequence being transferred, the sequence will be present in the host cell in a higher copy number than naturally occurring.

The term "operably linked" refers to the chemical fusion of two of more fragments of DNA in a proper orientation such that the fusion preserves or creates a proper reading frame, or makes possible the proper regulation of expression of the DNA sequences when transformed into plant tissue.

The term "expression" as used herein is intended to describe the transcription and/or coding of the sequence for the gene product. In the expression, a DNA chain coding for the sequence of gene product is first transcribed to a complementary RNA, which is often an mRNA, and then the thus transcribed mRNA is translated into the above mentioned gene product if the gene product is a protein. However, expression also includes the transcription of DNA inserted in antisense orientation to its regulatory elements. Expression, which is constitutive and possibly further enhanced by an externally controlled promoter fragment

thereby producing multiple copies of mRNA and large quantities of the selected gene product, may also include overproduction of a gene product.

The term "translation start codon" or "initiation codon" refers to a unit of three nucleotides (codon) in a nucleic acid sequence that specifies the initiation of protein synthesis.

The term "signal peptide" refers to the N-terminal extension of a polypeptide which is translated in conjunction with the polypeptide, forming a precursor protein and which is required for its entry into the secretory pathway. The signal peptide may be recognised by the mechanisms within the same species or unrelated species plants, necessary for direction of the peptide into the secretory pathway. The signal peptide may be active in roots, seeds, leaves, tubers and other tissues of the plant. The term "signal sequence" refers to a nucleotide sequence that encodes a signal peptide. The term "ER targeting signal" refers to the N-terminal extension of a polypeptide, which is translated in conjunction with the polypeptide forming a precursor protein and which is required for its entrance into the Endoplasmatic Reticulum (ER) of a cell. The ER targeting signal may be recognised by the mechanisms within the same species or unrelated species plants, necessary for direction of the peptide into the ER and/or the vacuole of a cell. ER targeting signals may be active in roots, seeds, leaves, tubers and other tissues of the plant. The term "ER targeting sequence" refers to a nucleotide sequence that encodes the ER targeting signal.

A "tissue specific promoter" refers to a sequence of DNA that provides recognition signals for RNA polymerase and/or other factors required for transcription to begin, and/or for controlling expression of the coding sequence precisely within certain

tissues or within certain cells of that tissue. Expression in a tissue specific manner may be only in individual tissues, or cells within tissues, or in combinations of tissues. Examples may include tissue specific expression in roots only and no other tissues within the plant, or may be in leaves, petals, ovules and stamen, and no other tissues of the plant. Here, "tissue specific" is also meant to describe an expression in a particular tissue or cell according to which the expression takes place mainly, but not exclusively, in the tissue. Such an expression is also termed "tissue abundant".

"Selective expression" refers to expression mainly, preferably almost exclusively, in specific organs of the plant, including, but not limited to, roots, leaves, tubers or seed. The term may also refer to expression at specific developmental stages in an organ, such as in early or late embryogenesis or in seedlings. In addition, "selective expression" may refer to expression in specific subcellular locations within the cell, such as the cytosol or vacuole.

The term "root specific nucleic acid sequence" refers to nucleic acid sequences, i.e. genes, coding sequences and/or regulatory elements which are exclusively or mainly active in roots of plants, in particular those which direct or contribute to a root abundant or root selective expression of a protein. The term "root abundant nucleic acid sequence" refers to nucleic acid sequences, i.e. genes, coding sequences and/or regulatory elements which are mainly active in roots of plants, in particular those which direct or contribute to a root abundant or root selective expression of a protein.

The present invention also relates to a vector comprising the nucleic acid sequences according to the above, in particular to a bacterial vector, such as a plasmid, or a virus.

In a particularly preferred embodiment of the present invention the nucleic acid sequences, i.e. the 5' and/or 3' regulatory elements of the present invention contained in the vector, are operably linked to a gene of interest which in this context may also be only its coding sequence, which may be a heterologous or autologous gene or coding sequence. Such a gene of interest may be a gene, in particular its coding sequence, conferring, for instance, disease resistance; drought resistance; insect resistance; herbicide resistance; immunity; an improved intake of nutrients, minerals or water from the soil; or a modified metabolism in the plant, particularly its roots. Such a modified metabolism may relate to a preferred accumulation of useful substances in roots, for instance sugars or, vice versa, in the depletion of substances undesirable in roots, for instance certain amino acids. Thus, in the context of the present invention, a gene of interest may confer resistance to infection by a virus, such as a gene encoding the capsid protein of the BWYV or the BNYVV virus, a gene conferring resistance to herbicides such as Basta®, or to an insecticide, a gene conferring resistance to the corn rootworm, a gene encoding the toxic crystal protein of Bacillus thuringiensis or a gene whose expression confers male sterility. A gene of interest includes also a coding sequence cloned in antisense orientation to the regulatory sequences directing its expression. Such a construct may be used specifically to repress the activity of undesirable genes in plant cells, in particular in roots, most preferably in primary, lateral seminal and/or crown roots. The gene of interest may also comprise signal sequences, in particular ER targeting sequences,

directing the encoded protein in the ER and eventually for instance in the cell wall, vascular tissue and /or the vacuole.

In a particularly preferred embodiment the vector defined above is comprised of further regulatory elements directing or enhancing expression of the gene of interest such as 5', 3' or 5' and 3' regulatory elements known in the art, for instance enhancers such as the 35S-enhancer. Regulatory elements considered in the present invention also encompass introns or parts of introns inserted within or outside the gene of interest.

In a particularly preferred embodiment of the present invention the 3' regulatory element is a transcription termination region, preferably a poly A addition or polyadenylation sequence, most preferably the poly A addition sequence of the NOS gene of Agrobacterium tumefaciens.

Thus, the nucleic acid sequences of the present invention are useful since they enable the root specific expression of genes of interest in plants, in particular monocotyledonous plants. Accordingly, plants are enabled to produce useful products in their roots. The nucleic acid sequences of the present invention may also be useful to regulate the expression of genes of interest depending upon the developmental stage of the transferred cell or tissue. Furthermore, the present invention allows the specific modification of the metabolism in roots.

In a particularly preferred embodiment of the present invention the vector furthermore contains T-DNA, in particular the left, the right or both T-DNA borders derived from Agrobacterium tumefaciens. Of course, sequences derived from Agrobacterium rhizogenes may also be used. The use of T-DNA sequences in the vector of the present invention enables the Agrobacterium tume-

faciens mediated transformation of cells. In a preferred embodiment of the present invention, the nucleic acid sequence of the present invention, together with the gene of interest and - optionally - further operably linked regulatory sequences, is inserted within the T-DNA or adjacent to it.

The present invention also relates to a non-biological, technical method of genetically modifying a cell by transforming it with a vector or a nucleic acid sequence according to the above, whereby the gene of interest operably linked to the nucleic acid sequence or sequences of the present invention is expressible in the cell. In particular, the cell being transformed by the method of the present invention is a plant, bacterial or yeast cell. In a particularly preferred embodiment of the present invention, the above method further comprises the regeneration of the transformed cell to a differentiated and, in a preferred embodiment, fertile plant.

The method to transform a cell with a vector according to the present invention may be any method known to effectively transfer nucleic acid sequences into a host cell, thereby allowing its expression therein and eventually the regeneration of the cell to a plant. In a particularly preferred embodiment of the present invention, the transfer of the nucleic acid sequence may be mediated by Agrobacterium tumefaciens or rhizogenes. However, any other method known to eventually achieve the same results may be used, such as direct uptake of nucleic acid sequences, microinjection of nucleic acid sequences, or particle bombardment.

The present invention also relates to host cells transformed with the vector of the present invention, in particular plant, yeast or bacterial cells, in particular monocotyledonous or di-

cotyledonous plant cells. The present invention also relates to cell cultures, tissue, calluses, etc. comprising a cell according to the above, i.e. a transgenic cell.

Furthermore, the present invention relates to plants, harvest and propagation material, parts of plants, plant tissue, plant seeds, plant leaves, plant roots, leaves, or embryos containing a host cell of the present invention. These plants, plant tissues, plant seeds and plant parts are also called "transgenic". These plants or plant parts are characterised by, as a minimum, the presence of the transferred nucleic acid sequence of the present invention in their genome or, in cases where the nucleic acid sequences are autologous to the transferred host cell, are characterised by additional copies of the nucleic acid sequences of the present invention. Preferably, the nucleic acid sequences of the present invention are linked to genes of interest or antisense DNA normally not found in the transferred cell operably linked to the nucleic acid sequence of the present invention, at least not in that order or orientation.

Thus, the present invention also relates to roots, leaves, calluses, plant tissue, tassels, etc., nonbiologically transformed, which possess, stably integrated in the genome of their cells, a heterologous or autologous nucleic acid sequence containing a regulatory element of the present invention recognised by the polymerases of the cells of said plant parts and the gene of interest encoding a protein of interest or being an antisense construct.

Thus, the invention relates to plants, nonbiologically transformed, which possess, stably integrated in the genome of their cells, a heterologous or autologous nucleic acid sequence containing a regulatory element of the present invention recognised

by the polymerases of the cells of said plants and a gene of interest encoding a protein of interest or being an antisense construct.

The teaching of the present invention is therefore applicable to any plant, plant genus, or plant species wherein the regulatory element of the present invention is recognised by the polymerases of the cells.

Finally, the present invention relates to a method for isolating or cloning a root specific gene and/or root specific regulatory elements, such as promoters whereby a nucleic acid sequence of the present invention is used to screen nucleic acid sequences derived from any source, such as genomic or cDNA libraries derived from plants, in particular monocotyledonous plants. The nucleic acid sequences of the present invention thereby provide a means of isolating related regulatory sequences of other plant species which confer root specificity to genes of interest operably linked to them.

Further preferred embodiments of the present invention are mentioned in the claims.

The invention may be more fully understood from the following detailed sequence descriptions which are part of the present teaching. The SEQ ID Nos. 1 to 15 are incorporated in the present invention. The positions indicated below refer to the sequence numbering of SEQ ID No. 14.

SEQ ID No. 1 is a partial DNA sequence of the zmGRP3 (glycine-rich protein: GRP) gene of Zea mays. The 1760 bp (base pair) sequence spans the region from and including position 1760 towards the 5' end, up to and including position 1.

SEQ ID No. 2 is a partial DNA sequence of the zmGRP3 gene of Zea mays. The 1348 bp sequence spans the region from and including position 1760 towards the 5' end, up to and including position 413.

SEQ ID No. 3 is a partial DNA sequence of the zmGRP3 gene of Zea mays. The 1096 bp sequence spans the region from and including position 1760 towards the 5' end, up to and including position 665.

SEQ ID No. 4 is a partial DNA sequence of the zmGRP3 gene of Zea mays. The 746 bp sequence spans the region from and including position 1760 towards the 5' end, up to and including position 1015.

SEQ ID No. 5 is a partial DNA sequence of the zmGRP3 gene of Zea mays. The 554 bp sequence spans the region from and including position 1760 towards the 5' end, up to and including position 1207.

SEQ ID No. 6 is a partial DNA sequence of the zmGRP3 gene of Zea mays. The 406 bp sequence spans the region from and including position 1760 towards the 5' end, up to and including position 1355.

SEQ ID No. 7 is a partial DNA sequence of the zmGRP3 gene of Zea mays. The 190 bp sequence spans the region from and including position 1760 towards the 5' end, up to and including position 1571.

SEQ ID No. 8 is a partial DNA sequence of the zmGRP3 gene of Zea mays. The 1354 bp sequence spans the region from and including

position 1354 towards the 5' end, up to and including position 1.

SEQ ID No. 9 is a partial DNA sequence of the zmGRP3 gene of Zea mays. The 1497 bp sequence spans the region from and including position 1497 towards the 5' end, up to and including position 1.

SEQ ID No. 10 is a partial DNA sequence of the zmGRP3 gene of Zea mays. The 1550 bp sequence spans the region from and including position 1550 towards the 5' end, up to and including position 1.

SEQ ID No. 11 is a partial DNA sequence of the zmGRP3 gene of Zea mays. The 1710 bp sequence spans the region from and including position 1710 towards the 5' end, up to and including position 1.

SEQ ID No. 12 is a partial DNA sequence of the zmGRP3 gene of Zea mays. The 856 bp sequence spans the region from and excluding the translation stop codon at position 2558 - 2560 towards the 3' end up to position 3416.

SEQ ID No. 13 is a partial DNA sequence of the zmGRP3 gene of Zea mays. The 440 bp sequence spans the region from and excluding the translation stop codon towards the 3' end up to position 3000.

SEQ ID No. 14 is a large part of the genomic DNA sequence of the zmGRP3 gene of Zea mays comprising 3416 nucleotides and encompassing the coding region of the gene, a sequence of 20 bp between and excluding the translation initiation codon ATG (position 1781 - 1783) and the nucleotides CCA at position 1758

- 1760 and the regulatory elements identified in SEQ ID Nos. 1 to 13.

SEQ ID No. 15 is a partial DNA sequence of the zmGRP3 gene of Zea mays. The 1074 bp sequence spans the region from and excluding the most 3' located base "C" at position 3416 of SEQ ID No. 14 to the sequence motif "TCC" at position 4488-4490. Thus, the complete genomic DNA sequence of zmGRP3 comprises 4490 bp.

The invention is further illustrated by way of example and the following drawings.

Figure 1 shows a restriction map of the genomic clone zmGRP3 described in SEQ ID No. 14.

Figure 2 shows restriction maps of 5' deletion constructs of the present promoter element corresponding to SEQ ID Nos. 1 to 7.

Figure 3 shows restriction maps of 3' deletion constructs of the present promoter element corresponding to SEQ ID Nos. 8 to 11.

Figure 4 shows a northern blot analysis for various tissues of Zea mays.

Figure 5 depicts DNA constructs used for transient expression experiments.

Example 1: Cloning of the genomic zmGRP3 sequence

The genomic clone zmGRP3 was isolated by differential screening of a cDNA library made from maize coleoptile nodes, subsequent

isolation of a specific root specific cDNA and isolation of the corresponding genomic clone.

A cDNA library from maize coleoptilar nodes with emerging developing young crown roots was made by choosing 7-9 days old wild-type maize seedlings. mRNA from 30-40 of the wild-type coleoptilar nodes (about 15 mg per piece of tissue) was isolated with oligo(dT)-cellulose using the "Quick prep mRNA purification kit" (Pharmacia, Freiburg). Double stranded cDNA was prepared using the "Time Safer"-cDNA synthesis kit of Pharmacia Freiburg. After elimination of cDNAs with a size less than 0.4 kb (kilobases) the cDNAs were ligated into the phage vector λ-ZAP-II (Stratagene) using the ligation kit "Gig-Pack-Gold" (Stratagene, Heidelberg). The phages were plated on E. coli strain XL 1B. The obtained library had a titre of 3.7 x 106 pfu/ml. The inserts had an average size of 1.4 kb. The phages of the cDNA library were plated out in low density (about 1000-2000 pfu per agar plate (12 x 12 cm)). Per plate two nitrocellulose filters as replicas were prepared. Both filters were then separately hybridized, each with a different probe.

As probes radioactively labelled first strand cDNAs of mRNA were used which a) were derived from the coleoptilar nodes of wild-type seedlings (with formation of crown roots) and b) derived from coleoptilar nodes of rt-cs mutant seedlings (without formation of crown roots) (Hetz, *et al.* Plant Cell, 1996. Pp. 10, 845-857). The first strand cDNA was labelled using radioactivity and, for further analysis, digoxigenin.

Radioactive Labelling of first strand cDNA:
Materials:
ca. 1-3 μg mRNA
5x RT-Buffer 250 mM Tris/HCl pH 8.5

	40 mM $MgCl_2$
	150 mM DTT
dNTP-Mix	dTTP, dGTP, dATP, ea 5 mM
	dCTP 1.3 mM

Random primer (0.8 μg/μl)
RNase-Inhibitor (30 U/μl)
32P-dCTP(10 μ Ci/μl)
SuperScript Reverse Transcriptase (Gibco, 100 U/μl)

For standard labelling 1-3 μg of mRNA were dissolved in 25.5 μl of H_2O, denaturated for 10 minutes at 65°C, then cooled in ice water. The following agents were added in order: 10 μl 5 x RT-Buffer, 1 μl of Rnasin, 2.5 μl of dNTP-Mix, 5 μl of random primer, 5 μl (=50 μCi) ^{32}P-dCTP, and 1 μl of SuperScript Reverse Transcriptase. The preparation was mixed thoroughly and incubated for 2 hours at 42°C. A final purification was done using the "Nucleotide Removal Kit" (Quiagen, Hilden).
Plaques giving a signal on the filter hybridized with the wild-type probe, but not giving a signal on the filter hybridized with rt-cs probe were excised and analysed. An in vivo excision was carried out and the cDNA insert was cloned into plasmid pBluescript. Altogether, 32 plaques were excised and analysed. For the further analysis, the isolated plasmid DNA clone was labelled with digoxigenin and hybridized in a northern blot analysis against 10 μg total RNA from wild-type and mutant coleoptilar nodes.

The labelling with digoxigenin of first strand-cDNA was done as follows:

Materials:
ca. 1-3 μg mRNA

5x RT-Buffer	250 mM Tris/HCl pH 8.5

	40 mM MgCl2
	150 mM DTT
dNTP-Mix	dCTP, dGTP, dATP, ea 5 mM
	dTTP 1.3 mM

RNase-Inhibitor (Fermentas, 30 U/μl)
SuperScript Reverse Transcriptase (Gibco, 100 U/μl)
Digoxigenin dUTP (Boehringer)
Oligo dT (0.6 μg/μl)
EDTA solution (0.2 M)
LiCl solution (4 M)
EtOH (100%, 70%)

The mRNA was denaturated for 5 minutes in 23.4 μl of H_2O at 65°C, then rapidly cooled in ice water. The following agents were added in order: 10 μl 5x RT-Buffer, 1 μl of RNase-Inhibitor (ca. 20 units), 5 μl of oligo-dT (final concentration 0.06 μg/ul) 3.6 μl of DIG-dUTP (final concentration 0.13 mM), 5 μl of dNTP-mix and 2 μl of SuperScript Reverse Transcriptase. The preparation was then incubated for 2 hours at 42°C. The labelled first strand cDNA was precipitated by adding of 2 μl of EDTA solution, 5 μl of LiCl solution and 130 μl of 100% EtOH. Following a washing with 70% EtOH, the cDNA resuspended in 50 μl of H_2O and stored at -20°C.

The northern analysis showed that the clone isolated hybridized with wild-type total RNA, but not with the mutant total RNA. As the mutant tissue differed from the wild-type tissue in the absence of crown roots, the expression of the isolated clone could be related to the formation of crown roots in maize seedlings. According to northern blot analysis, the clone was expressed exclusively in the cells of roots of young maize seedlings, in particular in the tissue. The clone was used to isolate a full-length cDNA clone. The full-length cDNA clone (zmGPR3 cDNA) was

in turn used to isolate a genomic clone zmGRP3 which is partially described in SEQ ID No. 14 as follows:

The sequence given in SEQ ID No. 14 comprises the 5' regulatory elements of the present invention, the coding region of the maize zmGRP3 gene and the 3' regulatory elements of the present invention except the most distal part of the 3' region which, however, is given in SEQ ID No. 15. The coding region of zmGRP3 spans from the translation initiation codon at position 1781 to 1783 to the translation termination signal TGA at 2558 to 2560. The 5' regulatory element of the present invention comprises at position 1712 up to 1719 the putative TATA-box "tataaata".

The sequences depicted in SEQ ID Nos. 1 to 7 comprise the putative TATA-box and are particularly useful for the purposes described in the present invention.

The 3' regulatory element of the present invention, in particular the elements described in SEQ ID No. 12 and 13 comprise at position 2957 the polyadenylation signal. The 3' regulatory element located 3' to SEQ ID No. 12 and 13 and depicted in SEQ ID No. 15 may prove particularly useful as expression enhancing element.

The coding sequence of zmGRP3 codes for a glycin-rich protein, whose transcripts accumulate exclusively in roots of young maize seedlings following developmentally specific patterns. The protein exhibits a hydrophobic domain at the N-terminal region followed by repeated glycin-rich motifs. The hydrophobic domain at the N-terminal region is believed to be part of an ER-type signal peptide. The obtained genomic clone is the first nucleic acid sequence encoding a glycin-rich protein expressed exclusively in the root system, in particular that of maize.

Example 2: Northern analysis

As the tissues to be compared, i.e. wild-type and mutant tissues differed only in the presence or absence of crown roots, the transcription of zmGRP3 mRNA could be identified to be closely connected with the formation of crown roots. However, to determine whether the zmGRP3 transcription could also be detected in other tissues or other types of roots, primary roots, lateral seminal roots, and lateral roots (of maize) the following northern analysis was carried out. mRNA was isolated from various organs such as kernels, shoot meristems, leaves, coleoptilar nodes, tassels, various zones of primary, lateral, seminal and crown roots and root tips of lateral roots. The mRNA was then on a gel and hybridized with the zmGRP3 cDNA isolated above.

Figure 4 A to D illustrate the detection of zmGRP3 transcripts in northern analysis. After electrophoretic separation and blotting, the RNAs (10 μg) were hybridized with the DIG-labelled zmGRP3 cDNA. Total RNAs were isolated from; Fig. 4A, coeloptilar node with emerging young crown roots (lane 1), shoot meristem (lane 2), root tips of crown roots (lane 3), of primary roots (lane 4), or of lateral seminal roots (lane 5), leaves (lane 6), kernel (12-14 days after pollination, lane 7), or tassel (lane 8). Fig. 4B, C and D represent RNA samples taken from defined root segments of crown roots (B), of primary roots (C), or of lateral seminal roots (D): root tip up to cm 1 (lane 1), cm 2 (lane 2), cm 3 (lane 3), and cm 4 (lane 4). Lane 5 in C represents root tips (up to 1 cm) of secondary roots. The lower parts of A, B, C and D show the ethidium bromide stained gels displaying the 18S and 26S rRNA bands.

Figure 4B indicates that zmGRP3 mRNA accumulation is developmentally and spatially regulated within the root with the highest

level of accumulation occurring in the meristematic and elongation region (cm 1), low levels in early regions of cell maturation (cm2) and again increasing levels in later regions of cell maturation (cm 3 and 4) (cm sections as defined in Held *et al*, Plant Physiol. (1993) pp. 102, 1001-1008). In crown roots the zmGRP3 mRNA is predominantly expressed in the root tip. A weak hybridization signal is detected in the later stage of root development (cm 4). A possible explanation for this difference in accumulation between crown roots and primary/lateral seminal roots is that the development of primary and lateral seminal roots begins 4 to 5 days before crown roots. The transcript was surprisingly not detected in tips of lateral roots.

Example 3: Preparation of transgenic plants

Materials and Methods:

Isolation and culture of immature embryos for particle bombardment

Immature maize embryos (crosses between inbred line A188 and H99) with a size of 1.0-1.5 mm were isolated under sterile conditions. They were cultured with the scutellar side facing upward on modified N6-Medium (D'Halluin *et al.* 1993) with 1 mg/l 2,4-D, solidified with 0.8% agarose or 0.3% phytagel.

The explants were bombarded 4 to 12 days after isolation. For the osmotic pre-treatment, the embryos were placed with the scutellar side facing upward on MSC-medium (Zhong *et al.* 1992) in which a higher osmotic value was adjusted with sucrose to 670 mOsm/kg. The osmotic treatment was started 4 h before bombardment and was continued for 20 to 24 h after bombardment. In ex-

periments where the rhizobial part of the embryo was bombarded, the embryo was cut immediately or one day after isolation.

Particle bombardment

Particle acceleration was performed with the PDS 1000/He gun (BioRad, Munich, Germany). Five µg plasmid DNA was precipitated onto gold particles with an average size of 0.4-1.2 µm (Heraeus, Karlsruhe, Germany) following a protocol described by BioRad (Munich, Germany) and modified according to Becker *et al.* (1994). For stable transformation experiments, the particle-DNA pellet was resuspended in 240 µl ethanol. For each bombardment, 3.5 µl were spread on the macrocarrier.

<u>Parameters used for particle bombardment</u>

Distance between	
A: rupture disk and macrocarrier	2.5 cm
B: macrocarrier and stopping screen	0.8 cm
C: stopping screen and target cells	5.5 cm
Gas pressure	1350 psi
Partial vacuum	28 inch Hg
Particles	gold (size: 0.4-1.2 µm)
Amount of particles	30 µg per shot

Constructs

In experiments for stable transformation, the p35Spat gene (Brettschneider et al., 1997) was used as selection marker gene. In co-transformation experiments 2.5 µg DNA of the plasmid with the selection marker gene was mixed with the second plasmid containing the promoter-reporter gene and precipitated together onto the particles. The promoter used for transient and stable

transformation experiments has the sequence depicted in SEQ ID No. 1 and was fused to a GUS reporter gene. Further deletion constructs in the promoter region were made which are depicted in Fig. 2 and 3.

Figure 2 depicts 5' deletion constructs (Fig. 2 (b)) of the full length promoter of SEQ ID No. 1 (Fig. 2(a)), while Figure 3 shows 3' deletion constructs (Fig. 3 (b)) of the full length promoter of SEQ ID No. 1 (Fig. 3 (a)).

In detail, Figure 2a is a schematic representation of the cloned promoter fragment of zmGRP3. The positions of the restriction sites of the following restriction enzymes are presented (the numbers denote the nucleotide positions of the restriction sites in the promoter fragment): *X: Xhol* (1), *G: Bgl II* (1206), *V: Pvul* (120), *K: Kpnl* (1354), *P:Pstl* (588), *N: BstNl* (572, 1760), *S: Sacl* (664), *B: BamHl* (698, 1014), *Z: BstXl* (412), *C:Clal* (1497, 1525, 1570), *D: Dral* (906), *Y: Xbal* (963). Furthermore, the position of the putative TATA box is indicated.

Figure 2b gives an overview of the 5' promoter deletion clones prepared. Presented is in each case the length of the promoter fragment still present (boldface), as well as the size of the region deleted from the 5' end (in brackets) of the promoter. The fragments were cloned in a vector that contained the ß-glucuronidase gene (uid A).

Figure 3a) is a schematic representation of the cloned promoter fragment of zmGRP3. The positions of the restriction sites of restriction enzymes are presented as in Fig. 2a.

Figure 3b) is an overview of the 3' promoter deletion clones prepared. Presented is in each case the length of the promoter

fragment still present (boldface), as well as the size of the region deleted from the 3' end (in brackets) of the promoter. The fragments were cloned in a vector that contained the ß-Glucuronidase Gen (uid A) downstream from the -46bp-region of the CaMV 35S promoter (with TATA box). Thus, these 3' deletion constructs contain the CaMV 35S promoter initiation site.

Expression studies

For transient expression studies, different maize tissues, i.e. roots, leaves of seedlings, germinating embryos, were bombarded with the promoter constructs identified above. For histochemical GUS-detection, the bombarded tissues were incubated one day after bombardment at 37°C for 20 h in X-gluc staining solution (McCabe *et al.* 1988). After staining leaf tissues with x-gluc chlorophyll was extracted in ethanol/acetic acid glacial (3:1) for 30 to 60 min at 65°C and washed once with 70% ethanol.

For quantification of the promoter strength, bombarded tissues were analysed in a protein GUS-assay (Jefferson *et al.* 1987). For standardisation of the transformation frequency, the promoter constructs were co-transformed with a *luciferase* reporter gene under control of the actin promoter from maize (positive control). As negative control, pUC 19 DNA was used in the same amount as the promoter constructs. One day after bombardment, proteins of the bombarded tissue were extracted and analysed for GUS and LUC activity. The analysed root tissue exhibited GUS and LUC activity, while the other bombarded tissues only showed LUC activity. These results clearly evidence root specificity of the promoter element of the present invention.

The table I below shows the results of the transformation of 5' deletion constructs.

Promoter	Root	Leaf	Embryo
SEQ ID No. 1, 0	+	-	-
SEQ ID No. 2, -412	+	-	-
SEQ ID No. 3, -664	+	-	-
SEQ ID No. 4, -1014	+	-	-
SEQ ID No. 5, -1206	+	-	-
SEQ ID No. 6, -1354	+	-	-
SEQ ID No. 7, -1570	+	-	-
negative control	-	-	-
positive control	+	+	+

Table I: Transformation of various maize tissues with 5' deletion promoter constructs.

All of the tested promoter constructs clearly and unambiguously show expression in roots, but not in leaves or in the embryo. Even the smallest promoter fragment (SEQ ID No 7, 190 bp length) shows significant expression exclusively in the root.

Figure 5 shows further gus-fusion constructs using the regulatory elements of the present invention (not to scale). The boxes marked "GUS" symbolise the coding region of the uidA gene from E. coli, the position of the start and stop codons is indicated in each case. The 35S promoter from pBI121 was deleted in the constructs produced (pBI121-35S) or replaced by the GRP3-promoter (pBI-GRP3-Prom, pBI121-GRP3-Prom-3') or the GRP3-promoter + 2x35S-Enhancer (pBI212-2x35S-En-GRP3-Prom-3'). In some cases moreover the 3' region of GRP3 (pBI121-GRP3-Prom-3', pBI121-2x35S-En-GRP-Prom-3') was inserted instead of the NOS-terminator. The positions of restriction endonuclease-sites relevant to this are indicated under the relevant constructs (H:

HindIII, L: Sal/I, X: XbaI, B: BamHI, M: SmaI, S: SacI, E: EcoRI).

Figure 5 thus shows further gus-fusions constructs using the regulatory elements of the present invention. PBI 121 is a positive control while PBI-121-35S is a negative control. PBI-121-GRP3-Prom is a construct using the promoter of SEQ ID No. 1 being functionally linked to a gus-reporter gene and the NOS-terminator. PBI-121-GRP3-Prom 3' is a DNA construct of the present invention using the promoter element of SEQ ID No. 1 functionally linked to the gus-reporter gene and a 3' regulatory element of the present invention having the sequence of SEQ ID No. 12 and added 3' thereto of SEQ ID No. 15. Therefore the 3' regulatory element spans the region from and excluding the translation stop codon at position 2558-2560 towards the 3' end up to position 4490. This 3' regulatory element being the combination of SEQ ID No. 12 and SEQ ID No. 15 has also been used in DNA construct PBI-121-2 X 35S-EN-GRP3-Prom-3' which is similar to the above described construct but additionally has the 2 X 35S-enhancer (nucleotide positions 7089 to 7357 and -346 to -78 of the CaMV virus genome relative to the start of transcription described in Fang et al. (1989) and Franck et al. (1980); data base accession No. V00141 Y 02048 VRL 12. Sept. 1993; NID: g 58821).

The following table II shows the expression results in transient essays in roots and leaves. Accordingly, it can be shown that both the 5' and 3' regulatory elements of the present invention contribute to tissue specific expression in roots indicating that DNA constructs using both the 5' and 3' elements of the present invention or the elements separately may prove useful in genetic engineering of plants.

Table II: Transient expression studies

Construct	Activity in the Transient Assay in Roots	Activity in the Transient Assay in Leaves
pBI121	+++++	+++++
pBI121-35S	-	-
pBI121-GRP3-Prom	+	-
pBI121-GRP3-Prom-3'	++	-
pBI121-2x35S-En-GRP3-Prom-3'	+++	-

(+: strong expression, degree indicated by number of "+", -: no or hardly any expression).

Explants were cultured after bombardment for two weeks on N6 1-100-25 medium at 26°C in the dark. Selection was started 14 days after bombardment when the explants were subcultured on N6 1-100-25 medium without casaminoacids supplemented with 5 mg/l phosphinotricin (PPT), the active compound of the commercial herbicide Basta®. After two weeks, the calli were subcultured for another two weeks on N6 1-100-25 medium with 5 mg/ l Basta®. For regeneration, embryogenic calli were transferred on hormonefree MS-medium with 1 mg/l PPT and cultured at 24°C under light (16 h).

Green shoots were transferred to Magenta boxes with half-strength MS-medium and 1 mg/l PPT solidified with 0.3 % phytagel. Plantlets with a height of 6-8 cm which survived selection were transferred into soil after they generated roots.
Small plants were cultured in soil in the greenhouse for 1 week under a plastic or glass lid. Routinely, more than 90% of the regenerants survived the transfer into soil. To confirm the selection, surviving plants were sprayed once or twice with a Basta®-solution containing 250 mg/l PPT and 0.1% Tween.

Transformants were verified by southern analysis

DNA isolation and Southern blot hybridisation

For the analysis of stable transformants, total genomic DNA was isolated from primary transformants and their progeny using the protocol of Dellaporta et al (1983). 10 or 15 µg of DNA, uncut or digested with restriction enzymes were separated by electrophoresis and transferred to a Hybond N membrane (Amersham/England) or a BiodyneA membrane (Pall/England). Introduced DNA of the present invention (SEQ ID No. 1) was detected using a modified protocol of the non-radioactive digoxigenin chemiluminescent method (Neuhaus-Url et al. 1993). Filters were hybridised with PCR-labelled Dig probes. Stably transformed transgenic maize plants could be obtained.

Example 4

In situ hybridization experiments of various types of maize roots with zmGRP

Method:
In situ hybridisation experiments were carried out according to a modified protocol from Coen et al., 1990 (Cell 63, 1311-1322). First the tissue to be analysed was fixed in 4% paraformaldehyd over night. The tissue then was dehydrated in an increasing concentration of ethanol and finally embedded in paraffin.
The tissue was cut in 8-12µm slices. After that the slices were hybridised with DIG labelled zmGRP3 RNA sense or antisense probes which had been hydrolytically digested to a size of about 200 bp. Hybridisation was carried out in 50% formamid at a tem-

perature of 50°C. Washing and the immunological staining was carried out exactly as described by Coen et al., 1990.

Results:

Longitudinal sections of different 5 day old root types were used. These slices were derived from primary root as well as from lateral crown roots and from stem derived crown roots. The embryogenic primary root as well as lateral and stem derived crown roots showed the same hybridisation pattern. In all cases there was no signal with the sense probe, whereas with the antisense probe there was a strictly root specific hybridisation. This hybridisation was cell specifically and was restricted to the epidermal cell layer and cells of the columella root cap. In all other tissue types there was no hybridisation signal.

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Coen et al., 1990 (Cell 63, 1311-1322).
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ART 34 AMDT

Gleiss & Große
Patentanwälte
München Stuttgart

Claims

1. A nucleic acid sequence for use in cloning and expressing a root specific nucleic acid sequence in a plant which is selected from the group consisting of

a) the nucleic acid sequence set out in SEQ ID No. 1 or a complementary strand thereof,

b) a nucleic acid sequence which has a degree of identity of more than 70 % to the nucleic acid sequence defined in (a) and

c) alleles of the nucleic acid sequence defined in (a) or (b).

2. The nucleic acid sequence of claim 1 which is selected from the group consisting of

a) the nucleic acid sequence set out in SEQ ID No. 2 or a complementary strand thereof,

b) a nucleic acid sequence which has a degree of identity of more than 70 % to the nucleic acid sequence defined in (a) and

c) alleles of the nucleic acid sequences defined in (a) or (b).

3. The nucleic acid sequence according to claim 1 or 2, which is selected from the group consisting of

a) the nucleic acid sequence set out in any one of SEQ ID No. 3 to 11 or a complementary strand thereof,

b) a nucleic acid sequence which has a degree of identity of more than 70 % to the nucleic acid sequence defined in (a) and

c) alleles of the nucleic acid sequence defined in (a) or (b).

4. A nucleic acid sequence for use in cloning and expressing a root specific gene in a plant which is selected from the group consisting of

a) the nucleic acid sequence set out in any one of SEQ ID No. 12 to 15 or a complementary strand thereof,

b) a nucleic acid sequence which has a degree of identity of more than 70 % to the nucleic acid sequences defined in (a) and

c) alleles of the nucleic acid sequence defined in (a) or (b).

5. The nucleic acid sequence of any one of the preceding claims, which is derived from maize.

6. A vector comprising the nucleic acid sequence of any one of the preceding claims.

7. The vector of claim 6, which is a bacterial or viral vector.

8. The vector of any one of claims 6 or 7, wherein the nucleic acid sequence of any one of claims 1 to 5 is operably linked to a gene of interest.

9. The vector of claim 8, wherein the gene of interest is chosen from a gene which confers a character of agronomic or industrial benefit.

10. The vector of claim 9, wherein the gene which confers a character of agronomic or industrial benefit is a gene for resistance to infection by a virus, a gene conferring resistance to a herbicide or to an insecticide or, alternatively, a gene whose expression confers male sterility.

11. The vector of any one of claims 6 to 10, which comprises further regulatory elements directing or enhancing the expression of the gene of interest.

12. The vector of any one of claims 6 to 11, wherein the further regulatory elements are 5',3' or 5' and 3' elements.

13. The vector of claim 12, wherein the 3' regulatory element is the poly A addition sequence of the NOS gene of Agrobacterium tumefaciens.

14. The vector according to any one of claims 6 to 13, which furthermore contains T-DNA.

15. The vector of claim 14, which contains the left, the right or both T-DNA borders.

16. The vector according to claim 14 or 15, wherein the nucleic acid sequence is located within the T-DNA or adjacent to it.

17. The vector according to claim 16, wherein the nucleic acid sequence in conjunction with the gene of interest is located within the T-DNA or adjacent to it.

18. A host cell containing the vector of any one of claims 6 to 17.

19. The host cell of claim 18, which is a plant, yeast or bacterial cell.

20. The host cell of claim 19, which is a cell from a monocotyledonous or dicotyledonous plant.

21. A cell culture comprising a cell according to any one of claims 18 to 20.

22. A plant comprising a host cell according to any one of claims 18 to 20.

23. Seeds, propagation material, harvest material and plant tissue, comprising a host cell according to any one of claims 18 or 20 or derived from a plant according to claim 22.

24. A method of genetically modifying a cell by transforming a cell with a nucleic acid sequence according to any one of claims 1 to 5 or a vector according to any one of claims 6 to

17, wherein the gene of interest contained in the vector is expressible in the cell.

25. The method of claim 24, wherein the cell is a plant, bacterial or yeast cell.

26. The method of claims 24 or 25, wherein the transformed cell is regenerated into a differentiated plant.

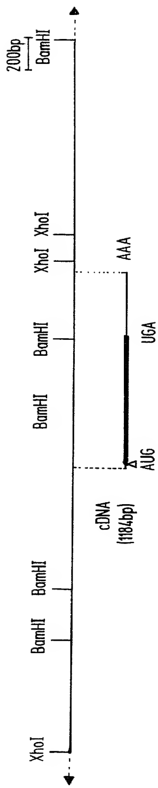
27. The method of any one of claims 24 to 26, wherein the cell is transformed by transfer of a vector or nucleic acid sequences from a bacterium to the cell.

28. The method of any one of claims 24 to 27, wherein the cell is transformed by direct intake of a nucleic acid sequence or a vector, by microinjection or particle bombardment with a nucleic acid sequence or a vector.

29. A method for isolating a root specific gene and/or root specific regulatory element from a plant, whereby a nucleic acid sequence of any one of claims 1 to 5 or a vector of any one of claims 6 to 17 is used to screen nucleic acid sequences derived from the plant.

Abstract

The present invention relates to regulatory elements for tissue specific, in particular root specific, expression of genes of interest in plants. The invention also relates to methods to obtain transgenic plants which show root specific expression of genes of interest and to methods for isolating root specific genes and/or root specific regulatory elements.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XhoI
XhoI
AAA
BamHI
UGA
BamHI
AUG
cDNA
(1184bp)
BamHI
BamHI
XhoI

Fig. 1

Fig.2

Fig.2a

Fig.2b

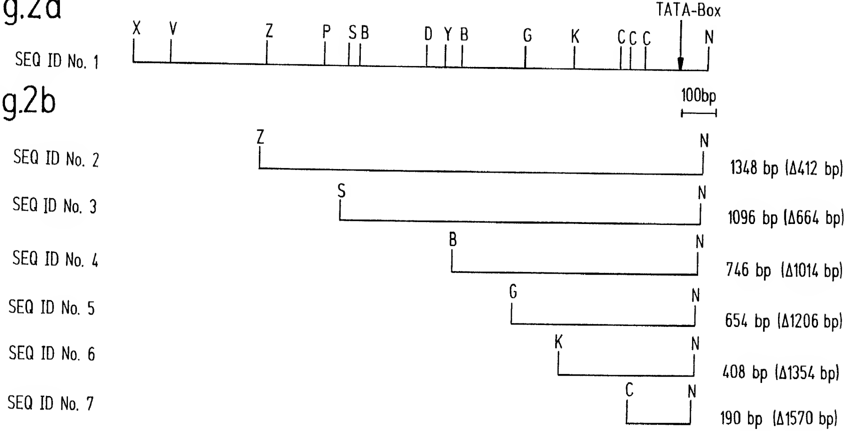


Fig.3

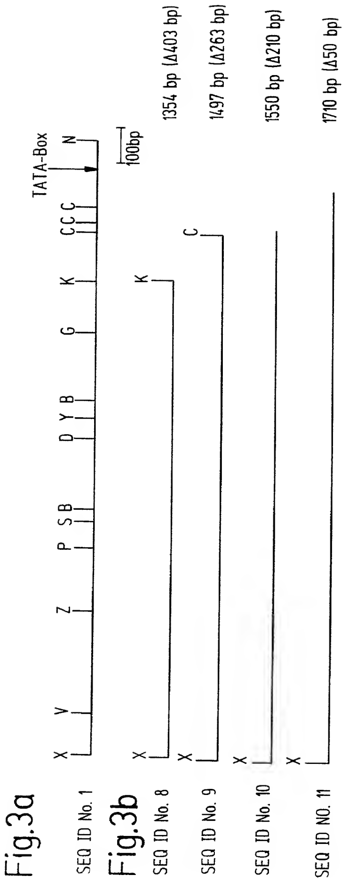
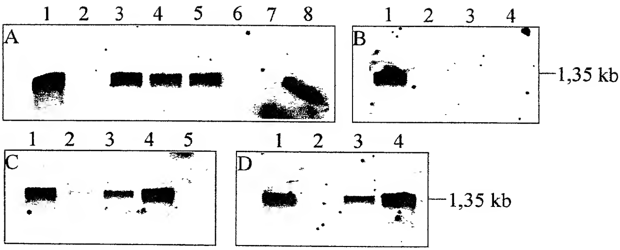


Fig.3a
SEQ ID No. 1
X
V
Z
P S B
D Y B
G
K
C C C
TATA-Box
N
100bp
Fig.3b
SEQ ID No. 8
X
K
1354 bp (Δ403 bp)
SEQ ID No. 9
X
C
1497 bp (Δ263 bp)
SEQ ID No. 10
X
1550 bp (Δ210 bp)
SEQ ID NO. 11
X
1710 bp (Δ50 bp)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A
1 2 3 4
B
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1 2 3 4 5
C
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D
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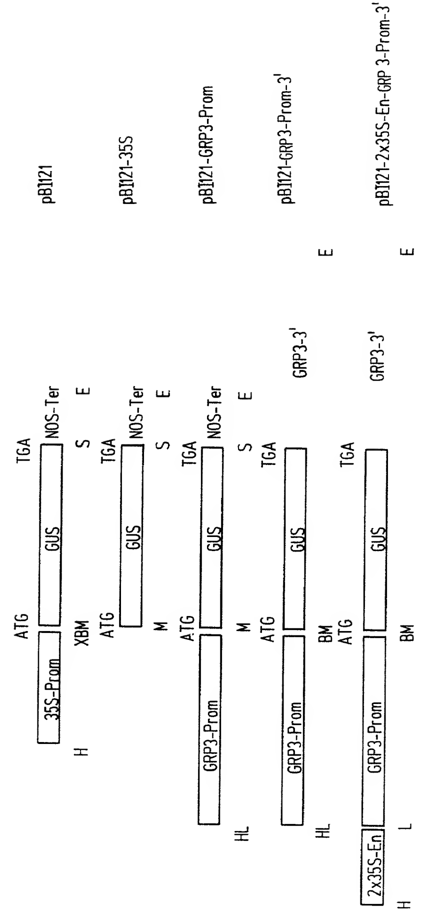
Fig.5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pBI121-GRP3-Prom-3'
pBI121-2x35S-En-GRP 3-Prom-3'
H
35S-Prom
ATG
XBM
GUS
TGA
NOS-Ter
S
E
ATG
GUS
M
TGA
NOS-Ter
S
E
HL
GRP3-Prom
ATG
M
GUS
TGA
NOS-Ter
S
E
HL
GRP3-Prom
ATG
BM
GUS
TGA
GRP3-3'
E
H
2x35S-En
L
GRP3-Prom
ATG
BM
GUS
TGA
GRP3-3'
E

23967

Declaration and Power of Attorney for Patent Application
Erklärung für Patentanmeldungen mit Vollmacht

German Language Declaration

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☒ wurde angemeldet am 10 September 1999 unter der US-Anmeldenummer oder unter der Internationalen Anmeldenummer im Rahmen des Vertrags über die Zusammenarbeit auf dem Gebiet des Patentwesens (PCT) PCT/EP99/06692 und am 4 December 2000 abgeädert (falls zutreffend)

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As a below named inventor, I hereby declare that:

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I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Regulatory Sequences for Root Specific Or Root Abundant Gene

Expression In Plants

the specification of which is attached hereto unless the following box is checked:

☒ was filed on 10 September 1999 as United States Application Number or PCT International Application Number PCT/EP99/06692 and was amended on 4 December 2000 (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

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Prior Foreign Applications
(Frühere ausländische Anmeldungen)

(Number) (Nummer)	(Country) (Land)	(Day/Month/Year Filed) (Tag/Monat/Jahr der Anmeldung)	Priority Not Claimed / Priorität nicht beansprucht
98117251.3	Europe	September 11, 1998	☐
			☐

Ich beanspruche hiermit Prioritätsvorteile unter Title 35, US-Code, § 119(e) aller US-Hilfsanmeldungen wie unten aufgezählt.

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(Application No.) (Aktenzeichen)	(Filing Date) (Anmeldetag)

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(Application No.) (Aktenzeichen)	(Filing Date) (Anmeldetag)	(Status) (patented, pending, abandoned) (Status) (patentiert, schwebend, aufgegeben)

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OIPE JC173 JUN 19 2001 PATENT & TRADEMARK OFFICE

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David W. O'Bryant 39,793 Frank W. Compagni, 40,567

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POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*

4

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Direct Telephone Calls to *(name and telephone number)*

1-00

Vor-und Zuname des einzigen oder ersten Erfinders **Günter Feix**	Full name of sole or first inventor **Günter Feix**
Unterschrift des Erfinders G. Feix Datum 4.5.01	Inventor's signature Date
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Staatsangehörigkeit **German**	Citizenship **German**
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Vor-und Zuname des zweiten Miterfinders (falls zutreffend) **Doerte ~~Wolf~~** Wulff	Full name of second joint inventor, if any **Doerte ~~Wolf~~**
Unterschrift des zweiten Erfinders Doerte Wulff Datum 4.5.01	Second Inventor's signature Date
Wohnsitz **Freiburg, Germany** DEX	Residence **Freiburg, Germany**
Staatsangehörigkeit **German**	Citizenship **German**
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2-00

(In Falle dritter und weiterer Miterfinder sind die entsprechenden Informationen und Unterschriften hinzuzufugen)

(Supply similar information and signature for third and subsequent joint inventors)

SEQUENCE LISTING

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Gene Expression in Plants

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